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14. ABSTRACT

The purpose of this contract is to carry out emerging infectious disease surveillance in Kenya. Specific areas in which work is performed include respiratory illness surveillance (particularly influenza), acute febrile illness surveillance, malaria resistance surveillance, diarrhea etiology and antimicrobial resistance surveillance, sexually transmitted illness surveillance, and capacity building. KEMRI maintained surveillance sites in both Kenyan Defense Forces and Ministry of Health clinics and hospitals throughout Kenya. KEMRI operated reference laboratories for this work in Nairobi, Kericho, and Kisumu, including the National Influenza Center (NIC), the arbovirus reference laboratory, the antimalarial resistance laboratory, entomology facilities, the Center of Excellence in Microscopy, the microbiology reference laboratory. Capacity development projects include continuation of a laboratory and medical maintenance student attachment program and a safety training program. The program was able to characterize respiratory viruses causing influenza-like illness in Kenya, determine etiologies of diarrheal illnesses and the antimicrobial resistance patterns of bacterial causes, determine the etiologies of sexually transmitted infections and acute febrile illnesses in military and civilian populations, and establish the pattern of antimalarial resistance across Kenya. An outbreak of dengue was investigated on the coast. Initial work to characterize leishmaniasis begun.

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INTRODUCTION:

KEMRI supports USAMRU-K's establishment of an emerging infectious disease surveillance network by providing contract personnel, laboratory and administrative facilities, capacity development capabilities for contracted personnel and partner organizations, regulatory oversight, and other required functions for the performance of infectious disease surveillance and research. The areas of research/surveillance performed are categorized by the pillars as defined by the US Department of Defense's Armed Forces Health Surveillance Center Department of Global Emerging Infectious Disease Surveillance and Response (DoD-GEIS). These pillars include respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

BODY: For clarity's sake, this report will be divided by DoD-GEIS pillar.

Respiratory Illness:

Global influenza surveillance to detect viral antigenic drifts and shifts must be reliably undertaken to protect public health. Sub-Saharan African countries have lacked laboratories and programs to conduct sustained influenza surveillance. To address this problem, USAMRU-K-GEIS and KEMRI developed a human influenza sentinel surveillance program at 8 civilian hospitals and 1Kenya military hospital since 2006. Influenza diagnostics is undertaken at the National Influenza Center (NIC) developed into a state-of-the-art BSL-2 laboratory through a significant monetary investment by GEIS. KEMRI also plays a significant role in assisting Kenya in Influenza Pandemic and other outbreak response.

445 NP swab specimens were received and processed at the NIC from ILI patients from the regular USAMRU-K sentinel surveillance network. 140 (32%) of the samples tested positive for influenza by RT PCR. Of these, 123 (88%) were influenza A and the rest were influenza B. When the 123 influenza A sample were subtyped, 75 (61%) were subtype H3N2 where as the remaining 48 (39% were subtype pH1N1. We did not observe any seasonal H1N1 cases. Thus seasonal influenza A H3N2 was the predominant strain circulating in Kenya. Of the 140 influenza positive samples, influenza viruses were obtained from 45 samples when the samples were inoculated in MDCK cells. This represented an overall isolation rate of 32%. The isolation rates differed according to the type and subtype of the influenza viruses in the patient sample. Thus, we obtained seven influenza B/Brisbane/60/2008-like viruses isolates representing an isolation rate of 41%, eleven isolates of pandemic H1N1 representing 23% and 27 isolates of seasonal influenza A H3N2 representing an isolation rate of 36 %. Unlike the previous reporting periods, we obtained the highest virus isolations rates for influenza A (H3N2). 36 non-influenza respiratory viruses were isolated. These included Adenoviruses (8%), RSV (6 %), Enteroviruses (20%), Parainfluenzavirus type 1 (42%), Parainfluenzavirus type 2 (6%) and Parainfluenzavirus type 3 (19%). Thus, overall majority (67%) of the non-influenza respiratory viruses isolated in this period were parainfluenza viruses. This is in contrast to the previous quarter in which RSV was the predominating non-influenza virus detected.

Acute Febrile Illness:

We have 23 pools of ectoparasites, 3 vials of endoparasites and 21 blood samples yet to be analyzed.

Leishmaniasis: This project began as a competitive project and has now been rolled in part into baseline activities. The lab has achieved most of its objectives by establishing six sand fly sampling sites in Kenya, one site in Ethiopia and four sites in Tanzania, and

identifying area that could pose the risk of leishmania transmission due to the presence of known competent vectors and etiological agents. Some of these sites within Kenya have been turned into regular sampling sites conducted in collaboration with the Acute Febrile Illness (AFI) studies also conducted by USAMRU-K. Our work in some of the more remote regions in Northern and North Eastern Kenya is paving the way for establishing new AFI surveillance sites in these areas in collaborationwith the Kenya Ministry of Health. Over the three year period of this study, a total of 24 field trips have been carried out in six sites in Kenya, four in Tanzania, and one in Ethiopia. Over 66,000 sand flies have been sampled, over 11,000 identified and over 45,000 tested by PCR for Leishmania infection. A total of 3,254 Phlebotomus sand flies were identified from all the sites. A total of 124 female sand fly pools have tested positive for Leishmania, and out of those, 12 pools have tested positive for either L. donovani or L. major. Of the 11 sites, at least 6 had never been sampled or studied for sand fly presence. These are Garissa and Lamu in Kenya and all four sites in Tanzania. In addition to testing for Leishmania infection, blood-meal analysis of blood fed females that had been sampled over the course of the 3 year sampling period began. So far, all blood fed females (1900) sand flies have been identified. These comprise of: 634 from Isiolo, 432 from Garissa, 632 from Wajir and 202 from West Pokot. From Isiolo, 325 (51.3%) of the total collection of blood fed sand flies are P. orientalis. 420 out of 634 blood-fed sand flies sampled in Isiolo have had DNA isolated and PCR amplification of the Cytochrome Oxidase 1 (CO1) gene complete. Approximately 80 of those had good results (band intensity) and are ready for DNA purification and sequencing. However, after several sequencing attempts, it was apparent that the target gene sequence could be very close to the sand fly or other non target vertebrate genome sequence thusinhibiting the detection of the blood meal DNA. The same difficulty was experienced by Muturi et al 2011 working on tse tse flies in Kenya. Currently, trial are being done using the cytochrome B gene as the target gene.

Dengue on the Coast: The Project was developed in 2011 and sent to the Ethical and Scientific Approval bodies. Meetings between Walter Reed and KDF personnel: WRP and KDF study investigators met in the 1st quarter to discuss the project activities and the way forward. This was done both at the Walter Reed and the Kenya Defense Forces offices and at the GEIS conference. The project was approved by the KEMRI Scientific and Ethical Approval committees, WRAIR scientific review in September/August 2012. Training of KDF Personnel: Training on Viral Hemorrhagic fevers and Arboviruses was conducted at the Moi Baraks in Eldoret by Walter Reed Scientists led by the Study P.I Dr. Rosemary Sang with support of other VHF staff. During the training, the military personnel were trained on how the Arboviruses and VHFs are transmitted, sensitized on the KDF protocol and taken through the consenting process. Those who consented to be involved in the study had blood samples collected from them for baseline screening purposes. A total of 125 soldiers from KDF consented to be involved in the study during this first exercise. The soldiers will leave by 1st November. When they come back, a post deployment sample will be taken from them during the post-deployment training.

Malaria:

In vitro drug sensitivity: In the FY12, 394 P. falciparum specimens collected from 5 sites, 3 in western Kenya and 2 sites in Eastern Kenya were tested for susceptibility against 12 antimalarial drugs using SYBRGreen 1 assay technique. Samples from nearby sites, approximately 15 minutes drive from the lab were assayed by immediate ex vivo while those from far placed sites tested along with 2 index Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)] were first cultured to adapt to in vitro replication prior to testing. Drugs tested include chloroquine (CQ), quinine (QN), artemisinin (AR), amodiaquine (AQ), artemether (AT), lumefantrine (LU), atovaquone (AV), tafenoquine (TQ), dihydroartemisinin (DHA), Piperaquine (PPQ), Mefloquine (MQ) and doxycycline (DX). DHA and PPQ were additional drugs included to drug panel dueto priority changes by the Kenya Ministries of Health and the

scientific/public health consensus of intended use of Duo Cotecxin in place of coartem in future.

A total of 66 isolates, 58 from Kisumu and 8 from Kisii district hospitals were assayed. Isolated from distant sites requiring culture adaptation prior to assaying were mostly not tested due to delays in approval of our blood collection protocol by the KEMRI- ERB. The protocol is now in place and blood from 1st October 2012, and drug testing back on track. In vitro activities of policy recommended drug Coartem® partner drugs artemether appear to be stable within the reported ranges (Akala et al., 2011), while lumefantrine median IC50 has risen marginally suggestive of tolerance. Continued surveillance of drug responses in vitro will soon be complemented with in vivo efficacy trial for the policy drug, Coartem to assess the effect of risen IC50 on the actual activities in the field.

Molecular assays: In the FY 2012 a total of 356 P. falciparum specimens were collected; 173 from Kisumu, 88 from Kisii, 29 from Kericho and 66 from Malindi District Hospitals. Polymerase Chain reaction (PCR) was performed on these samples to assess for mutations at codons 86, 184, 1034 and 1042 of the PfMDR1 gene using real-time PCR/allelic discrimination and Conventional PCR was used to assess mutations at position 76 of the PfCRT gene in all the collected samples. As per the data represented in tables 2 and 3 of the molecular section below, there is increased prevalence of pfmdr1 wild type 86N genotype that is indicative of increasing tolerance of plasmodium falciparum towards mefloquine, lumefantrine and artemisinin and its derivatives within the Kenyan population. Similarly, it has been suggested that this genotype change partly confers susceptibility to chloroquine. This suggestion bodes well with our in vitro data that shows lower median IC50, and percent resistance compared to previous years.

Mortality and morbidity due to malaria remain considerable in sub-Saharan Africa. Data suggest clinical malaria is more likely to develop in HIV-infected individuals than in those uninfected. In addition to the effects on malaria disease, the widespread use of trimethoprim/sulfamethoxazole prophylaxis (TMP/SMX) in HIV-infected individuals may place populations at risk of developing significant cross-resistance to antimalarials. There is increasing interest in determining whether daily TMP/SMZ use is necessary following the initiation of antiretroviral therapy (ART) and resultant immune reconstitution. Importantly, the effect of discontinuing TMP/SMZ prophylaxis on malaria will likely drive policy recommendations for Kenya and WHO in sub-Saharan Africa. Given globally, 22 million people are infected with HIVwith the majority living in sub-Saharan Africa (SSA), there are over 10million individuals in SSA on a daily antibiotic. This has enormous implications on drug resistance. We propose to examine the effect of discontinuing TMP/SMZ prophylaxis in immune reconstituted HIV-infected individuals on malaria incidence, parasitemia and drug resistance. The larger randomized control cohort began enrolling 1 Feb and has completed enrollment (n=500) as of 6 August. Samples are being kept at UW facilities until WRAIR IRB approval is finalized.

The heightened sense of awareness and interest in malaria is not only driven by the number of mortalities seen, but also by a concern that malaria may reach greater epidemic proportions due to multidrug resistant parasites. USAMRU-K will conduct a surveillance study in Western Kenya that seeks to determine the resistance of P. falciparum to Artemisininbased combination therapy by combining in vivo and in vitro methods. Patients aged between 6 months and 65 years who meet eligibility criteria will be recruited. Directly observed treatment will be administered and the subjects followed up for 42 days. P. falciparum drug sensitivity testing will be done against a range of antimalarial drugs, alone or in combination using the Malaria SYBR green assay. PCR on dried blood spots or fresh blood samples will be used to assess established P. falciparum genetic markers of drug resistance and to do DNA fingerprinting. Samples may be shipped to overseas reference labs for assistance in identification of new markers. Samples will be shipped in batches to WRAIR and/or an overseas reference lab for pK analysis. Data on existing and emerging parasite anti-malarial

resistance that this study will generate may assist with military and public health policy setting as well as prioritization of malaria product development. Additionally, this data will contribute to the pool of data regarding antimalarial resistance collected from other malaria endemic areas and may help in formulating global policies regarding malaria treatment.

Accurate diagnosis of malaria infections is critical for clinical, epidemiological and research purposes. Microscopy remains the most preferred diagnostic methods despite its shortcomings. Alternative methods of malaria diagnosis continue rise but their development and performance evaluation is constrained by lack necessary Plasmodium species and parasite densities representing different epidemiological distribution of the disease. Improving proficiency in microscopic diagnosis and development of reliable alternative diagnostics can only be achieved with properly characterized parasitized and non-parasitized blood samples and establishment of a sample repository. 517 potential volunteers tested for malaria, 137 positive cases detected with P. falciparum 111, P. malariae 3, P. ovale 3, P. falciparum + P. malariae 15, P. falciparum + P. ovale 3 and P. falciparum + P. malariae + P. ovale 2. 62 positive blood samples have been collected, P. falciparum 43, P. malariae 2, P. ovale 2, P. falciparum + P. malariae 9, P. falciparum + P. ovale 5 and P. falciparum + P. malariae + P. ovale 1. Approximately 17,000 malaria blood films, 900 whole blood aliquots and 250 dried filter paper blood spots prepared from the samples collected. Blood films prepared have been used to support 9 microscopy training courses with 146 participants from clinical and research institutions benefiting.

Enterics:

Acute gastroenteritis is a debilitating disease and is considered a major disease non-battle injury for deployed U.S. military personnel. A clinical surveillance protocol (WRAIR #1549) to identify microbial pathogens from human stool specimens collected at sites within the GEIS network in Kenya is currently being conducted at the Microbiology Hub Kericho (MHK), USAMRU-Kenya. This protocol is a case (volunteers with acute diarrhea) control (volunteers with no diarrhea) study that allows for the collection of stool specimens recruited at an outpatient clinical setting. Briefly, stool specimens are collected in preservation media at the surveillance sites and transported to the MHK where they are processed and tested for bacterial, parasitic, and viral pathogens. Enteric bacteria identification and antibiotic susceptibility are conducted. Ova and cysts of parasites are identified by general and immunofluorescence microscopy. Enteric viruses are diagnosed using either an enzyme immunoassay or PCR. The greatest benefit to the DoD is having a highly competent microbial disease clinical laboratory that will provide much needed support to the AFRICOM mission. Scientifically, the enterics surveillance conducted is providing valuable data on the prevalence of enteric pathogens in Kenya as well as potential patterns of antibiotic resistance among bacterial isolates in Kenya.

A total of 389 stool samples (299 cases and 190 controls) were received and processed for enteric bacterial, parasitic and viral pathogens during the 4th quarter FY12 from 8 surveillance sites. 173 enteric pathogens were detected from both the cases and controls. 69% of the total pathogens were protozoan, 14% viral, 15% bacterial and 2% helminth. Shigella spp was the most prevalent bacterial pathogen (46% of all bacterial isolates) followed by Campylobacter spp. Shigella spp continues to be most prevalent in the bacterial pathogen from most sites. The backlog of archived isolates for the detection of the diarrheagenic E. coli is being conducted now. There was backlog of isolates due to ordering and delivery delays with reagents and primers. Antibiotic susceptibility testing of the Shigella isolates showed multidrug resistance to common antibiotics that are commonly prescribed for treatment of diarrhea. Of Shigella isolates, 58% were resistant to tetracycline, trimethoprim/sulfamethoxazole, and ampicillin. All Shigella and Salmonella spp. isolates were susceptible to ciprofloxacin with 100% of the Salmonella spp. isolates susceptible to

tetracycline and trimethoprim/sulfamthoxazole. Blastocystis hominis was the most common protozoan parasite among all surveillance sites. In the literature, it is classified as non-pathogenic, but it has been detected enough that the laboratory is reporting its identification to the sites. Entamoeba histolytica/dispar and Giardia lamblia were the next two most common parasites. Isiolo District Hospital located in Isiolo was activated as a site on 30 July 2012. The laboratory for the Kenyan Defence Force Eldoret site was renovated in the 4th quarter of FY12 and upon IRB approval will be activated for sample collection. The Microbiology Hub Kericho became College of American Pathologists (CAP) certified in May of 2012. The laboratory continues to conduct internal audits in order to prepare for future inspections. As the menu for enteric viruses has been limited to Rotavirus in the current surveillance, a new multiplex PCR assay for the simultaneous detection of Norovirus (GI and GII), Astrovirus, Adenovirus and Rotavirus has been optimized in the laboratory. The implementation of this new PCR assay was delayed due to procurement but will be implemented in FY13 for enteric virus detection in the laboratory.

Sexually Transmitted Infections:

In Kenya, one of the more prosperous countries in East Africa, patients presenting to Ministry of Heath clinics with complaints suggestive of STIs (discharge or genital ulcer) often go undiagnosed, and are treated empirically with broad spectrum antibiotics. The drug resistance profiles, especially of gonorrhea, is largely unknown. In partnership with MOH and the KDF, all patients presenting to Kisumu and the Mbagathi District Hospital, the Mutwonge Naval base, Lanet Military barracks clinic and Kahawa barracks clinic with symptoms suggestive of gonorrhoea are offered anonymous screening for gonorrhea and chlamydia (GC). Specimens are taken for detection and isolation of Neisseria gonorrhoeae. Treatment is provided as per the ministry of Public Health and Sanitation guidelines. Antimicrobial susceptibility is determined using the E test method. A grand total of 70 individuals were screened for eligibility and 45 elligible subjects participated in the study. 7(15.6%) of the 45 were gram negative bacteria culture positive. However, only 5(12%) of the 7 culture positives were confirmed to be Neisseria gonorrhoeae which were subsequently tested for antimicrobial susceptibility to a selection of antimicrobial agents using the E test method. None of the bacteria isolates were found to be resistant to extended-spectrum chephalosporins tested(cefixime and ceftriaxone), Azithromycin or Spectinomycin. However, 1(20%) was found to be resistant to Ciprofloxacin while 3(60%) were resistant to Tetracycline.

Capacity Development:

A new epidemiological study that seeks to assess the baseline incidence rates of non-communicable and non-traumatic serious adverse events (NC/NT-SAE) and of adverse events of specific interests in catchment areas of the Phase III trial of the candidate malaria vaccine RTS, S/AS01E was awarded to Kombewa research Centre. The study will heavily rely on the HDSS platform for implementation. The study projected date is early 2013 and is sponsored by GSK. At the request of the Kenya Civil Registration Department (CRD), the KWHDSS will partner with the Kenyan government, to improve registration of vital events (Birth and Deaths) within the catchment area. The project will be funded by the INDEPTH network and is a potential source of additional funding for the KWHDSS. The first round of data updates was initiated in July 2012. Preliminary results are highlighted under the result section. The current survey is expected to run till end of November. Data cleaning and reconciliation will be done in the month of December before the 2nd round of updates commences in January 2013.

The student attachment program emphasizes hands on training experience for students in a professional environment. All interns undergo laboratory rotation in three laboratories in Nairobi handling arboviruses, respiratory viruses and sexually transmitted infections (STI). The training schedule comprises of three months training in specialized laboratory techniques used in the identification and characterization of emerging and re-emerging

infectious diseases under the supervision of technical staff and scientists. During their internship students also get trained on laboratory safety, biosecurity, quality assurance and quality control. The program ensures that students' progress is systematically monitored and that student support systems are available. The internship program provides undergraduates and diploma students' practical training and research experiences to strengthen their knowledge and skills, It also enables undergraduate students to carry out research projects which is part of their university curriculum in order to graduate. This leads to gainful employment and less training is needed for the new employee. As part fostering collaboration between the Kenya Defense Forces (KDF) and KEMRI/USAMRU-K, the internship program also admits nominated KDF laboratory personnel for hands-on training in the GEIS laboratories. A total of 38 students completed internships under this program. Due to increased awareness, the program has become extremely competitive. Currently approximately 40-50 applications are received monthly from local colleges and universities and from our neighboring countries. There is a scheduled in-take of new students with well co-ordinated interviews. Incorporation of the human resources department has improved the overall running of the program by ensuring that the country labor laws are adhered to and liability issues are taken care of.

KEY RESEARCH ACCOMPLISHMENTS:

Characterization of etiologies of influenza-like illness in Kenya

Identification of circulating strains of Influenza virus in Kenya

Characterization of selected viral, bacterial, and rickettsial etiologies of febrile illness in Kenya

Determination of leishmania prevalence in sand flies in select regions of Kenya

Response to dengue outbreak in coastal Kenya

Continued elucidation and tracking changes in antimalarial resistance patterns in Kenya

Training of Ministry of Health microscopists in accurate, reliable malaria microscopy

Ongoing characterization of etiologies of diarrheal illnesses in Kenya

Determination of prevalence of GC and Chlamydia among individuals seeking care with symptoms of STI

Human and infrastructure capacity development programs

REPORTABLE OUTCOMES: See references.

CONCLUSION:

KEMRI provides critical support to USAMRU-K's emerging infectious disease surveillance program in Kenya. Without KEMRI, USAMRU-K would not be able to execute its mission. KEMRI provides the legal and regulatory framework, personnel, and laboratory structure necessary to carry out scientific work. The organizations exist in partnership, with USAMRU-K working fully under the KEMRI umbrella in Kenya. Together, we have made great strides in establishing surveillance capabilities in the areas of respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

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